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Received 4 October 2004  
Accepted 5 December 2005  
Online 16 December 2005

# Crystallization and preliminary X-ray analysis of PH1566, a putative ribosomal RNA-processing factor from the hyperthermophilic archaeon *Pyrococcus horikoshii* OT3

A putative ribosomal RNA-processing factor consisting of two KH domains from *Pyrococcus horikoshii* OT3 (PH1566; 25 kDa) was crystallized by the sitting-drop vapour-diffusion method using PEG 3000 as the precipitant. The crystals diffracted X-rays to beyond 2.0 Å resolution using a synchrotron-radiation source. The space group of the crystals was determined as primitive orthorhombic  $P2_12_12_1$ , with unit-cell parameters  $a = 45.9$ ,  $b = 47.4$ ,  $c = 95.7$  Å. The crystals contain one molecule in the asymmetric unit ( $V_M = 2.5$  Å<sup>3</sup> Da<sup>-1</sup>) and have a solvent content of 50%.

## 1. Introduction

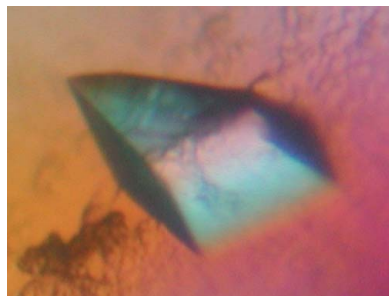
The biogenesis of ribosomal subunits in eukaryotes includes the synthesis, maturation and assembly of four ribosomal RNAs (rRNAs) and ~75 ribosomal proteins (Eichler & Craig, 1994; Kressler *et al.*, 1999; Venema & Tollervey, 1999; Lalev *et al.*, 2000). rRNAs are matured by successive endo- and exoribonucleolytic cleavages of pre-rRNAs (Kressler *et al.*, 1999; Venema & Tollervey, 1999) by rRNA-processing factors that contain one or more KH domains, which are a type of RNA-binding domain (Burd & Dreyfuss, 1994).

The rRNA-processing factor Dim2p is a core constituent of small ribosomal units in yeast nuclear 40S pre-ribosome as well as seven other major non-ribosomal proteins: Rrp12p, Tsr1p, Enp1p, Hrr25p, Nob1p, Dim1p and Rio2p (Schafer *et al.*, 2003). Dim2p is required for pre-rRNA processing at cleavage sites A<sub>1</sub> and A<sub>2</sub> and for 18S rRNA dimethylation (Vanrobays *et al.*, 2004) and its homologs have been found from archaea to metazoans. However, only one tertiary structure has been determined thus far for Dim2p-homologous proteins: the crystal structure of APE0754 from the aerobic hyperthermophilic archaeon *Aeropyrum pernix* (R. Zhang, T. Skarina, A. Savchenko, A. Edwards & A. Joachimiak, unpublished results). Here, we report the crystallization of another Dim2p homologue, PH1566 from the anaerobic hyperthermophilic archaeon *Pyrococcus horikoshii* OT3, which has 26 and 34% amino-acid sequence identity with Dim2p and APE0754, respectively.

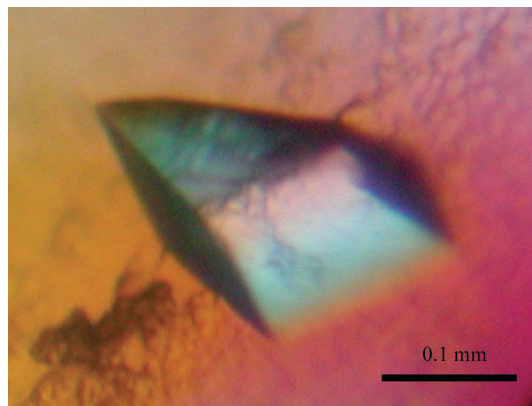
## 2. Methods and results

### 2.1. Expression and purification

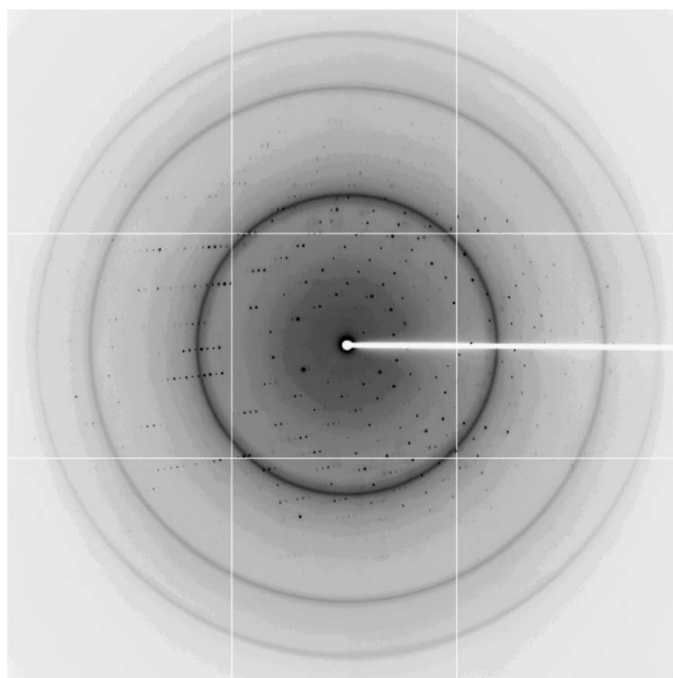
The gene of the Dim2p homologue (PH1566) was identified in the *P. horikoshii* OT3 genome, amplified by PCR and transformed into the expression vector pET-28a (Novagen). Recombinant PH1566 with an N-terminal His tag was overexpressed in *Escherichia coli* strain Rosetta (DE3) (Novagen). Cells were harvested by centrifugation, resuspended in lysis buffer (50 mM Tris-HCl pH 8.5, 0.3 M NaCl) and disrupted by sonication. The *E. coli* lysate was then incubated at 343 K for 30 min. After centrifugation, PH1566 was purified from the supernatant using Ni Sepharose 6 Fast Flow (Amersham Biosciences). After the removal of the His tag by thrombin digestion, PH1566 was further purified by two column chromatography steps: Resource Q 6-ml and Superdex 200 HR 10/30 (Amersham Biosciences).



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**Figure 1**  
A crystal of PH1566 grown at 293 K using PEG 3000 as precipitant.



**Figure 2**  
A diffraction image (1° oscillation) of the PH1566 crystal. The edge of the diffraction image corresponds to a resolution of 1.8 Å. The data were collected on BL41XU at SPring-8.

## 2.2. Crystallization

Crystallization trials were performed by the sitting-drop vapour-diffusion method using crystallization screening kits: Crystal Screens 1 and 2 (Hampton Research) and Wizard I and II (Emerald Bio-structures). Crystals appeared in the presence of polyethylene glycol (PEG) 3000 as the precipitant. After refinement of the crystallization conditions, crystals suitable for X-ray analysis were obtained in one week by mixing 1.0 µl of the protein solution (20 mg ml<sup>-1</sup> in 50 mM Tris-HCl pH 8.5, 0.05 M NaCl) and 1.0 µl of a reservoir solution consisting of 9% (w/v) PEG 3000, 100 mM cacodylate buffer pH 6.2 and 0.2 M MgCl<sub>2</sub>. A drop was equilibrated against 500 µl reservoir solution at 293 K. Fig. 1 shows a typical crystal (0.2 × 0.1 × 0.1 mm).

**Table 1**

Crystal parameters of PH1566.

Values in parentheses are for the highest resolution shell.

X-ray source	SPring-8 BL41XU
Wavelength (Å)	1.000
Space group	<i>P</i> <sub>2</sub> <sub>1</sub> <sub>2</sub> <sub>1</sub> <sub>2</sub> <sub>1</sub>
Unit-cell parameters (Å)	<i>a</i> = 45.9, <i>b</i> = 47.4, <i>c</i> = 95.7
Resolution range (Å)	50.0–2.00 (2.07–2.00)
Observed reflections	99494
Unique reflections	14815
Data completeness (%)	97.9 (99.2)
Redundancy	3.8 (3.5)
<i>R</i> <sub>merge</sub> <sup>†</sup>	0.056 (0.245)
<i>I</i> / <i>σ</i> ( <i>I</i> )	39.9 (4.4)

<sup>†</sup>  $R_{\text{merge}} = \sum_{hkl} \sum_i |I_i(hkl) - \langle I(hkl) \rangle| / \sum_{hkl} \sum_i I_i(hkl)$ , where  $I_i(hkl)$  is the *i*th intensity measurement of reflection *hkl*, including symmetry-related reflections, and  $\langle I(hkl) \rangle$  is its average.

## 2.3. X-ray data collection and processing

The crystal of PH1566 was picked up in a nylon loop (Hampton Research), transferred to a cryoprotective solution containing 20% (v/v) MPD, 7% (w/v) PEG 3000, 80 mM cacodylate buffer pH 6.2 and 0.16 M MgCl<sub>2</sub> and then mounted for flash-cooling at 100 K using a Rigaku cryostat. Diffraction data were collected at beamline BL41XU at SPring-8 (Harima, Japan) using an ADSC Quantum 315 detector. The wavelength was set to 1.000 Å and the crystal-to-detector distance was 250 mm. The crystals diffracted to beyond 2.0 Å resolution (Fig. 2). The diffraction data were indexed and scaled with *HKL2000* (Otwinowski & Minor, 1997). The space group of the crystals was determined to be *P*<sub>2</sub><sub>1</sub><sub>2</sub><sub>1</sub><sub>2</sub><sub>1</sub>, with unit-cell parameters *a* = 45.9, *b* = 47.4, *c* = 95.7 Å. The crystals contain one protein molecule per asymmetric unit according to the Matthews coefficient (*V*<sub>M</sub> = 2.5 Å<sup>3</sup> Da<sup>-1</sup>; Matthews, 1968). The data statistics are given in Table 1.

Structural determination by molecular replacement using the coordinates of APE0754 (PDB code 1tua; R. Zhang, T. Skarina, A. Savchenko, A. Edwards & A. Joachimiak, unpublished results) as a search model is currently under way.

The synchrotron-radiation experiments were performed at BL41XU in SPring-8 (Harima, Japan) with the approval of the Japan Synchrotron Radiation Research Institute (Proposal No. 2005A0820-NL1-np-P3K). This work was supported in part by the National Project on Protein Structural and Functional Analyses of the Ministry of Education, Culture, Sports, Science and Technology of Japan and by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

## References

- Burd, C. G. & Dreyfuss, G. (1994). *Science*, **265**, 615–621.
- Eichler, D. C. & Craig, N. (1994). *Prog. Nucleic Acids Res. Mol. Biol.* **49**, 197–239.
- Kressler, D., Linder, P. & de la Cruz, J. (1999). *Mol. Cell. Biol.* **19**, 7897–7912.
- Lalev, A. I., Abeyrathne, P. D. & Nazar, R. N. (2000). *J. Mol. Biol.* **302**, 65–77.
- Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
- Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.
- Schafer, T., Strauss, D., Petfalski, E., Tollervey, D. & Hurt, E. (2003). *EMBO J.* **22**, 1370–1380.
- Vanrobays, E., Gélugne, J., Caizergues-Ferrer, M. & Lafontaine, D. L. J. (2004). *RNA*, **10**, 645–656.
- Venema, J. & Tollervey, D. (1999). *Annu. Rev. Biochem.* **33**, 261–311.